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INHIBITION OF RABBIT BRAIN MONOAMINE OXIDASE BY TRANS-DICHLOROTETRA (PYRIDINE DERIVATIVES) OF COBALT (III) COMPLEXES.

By

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Abstract :

Rabbit brain monoamine oxidase (MAO) is rapidly inactivated when incubated with trans-$[Co(3\text{-}R\text{-}pyridine)_4Cl_2]Cl$ since R is methyl (CMPC) or ethyl (CEPC) group. These complexes inhibit MAO enzyme with time dependent and concentration dependent response with I_{50} value equal to $8.3x10^{-11}$ & $3x10^{-11}$ M respectively. $8.3x10^{-10}$ M from CMPC & CEPC inhibited the MAO enzyme by a noncompetitively inhibition type. The values of K_m and V_{max} were determined from the lineweaver-Burk plot. The MAO enzyme is inhibited also by methyl pyridine which is a precussor of these complexes. This inhibition increased by increasing the concentration of methyl pyridine.

Introduction

Monoamine oxidase (MAO) [monoamine=O_2 oxidoreductase (deaminating) (flavine-containing) EC 1.4.3.4] is responsible for the metabolism of biogenic amines in the peripheral and

central nervous system and is therefore important in the etiology of affective disorders, (Balsa et al 1987). Multiple forms of MAO exist in mammalian liver and brain which have been characterized by their sensitivity to inhibitor drugs and their specificity for substrates. The MAO inhibitor drugs are indicated for the treatment of some forms of mental depression as well as for some forms of hypertension. There have been reports of the endogenous activators (Demish et al 1983) or inhibitors (Becker et al 1983) of MAO in the tissues which may be important in some psychiatric conditions. The uptake of MAO inhibitors may exerts their effect in the central nervous system (CNS) by increasing the concentration of neurotransmitters in the synaptic cleft, therefore increasing the duration of action of the neurotransmitter as well as the intensity of post-synaptic receptor stimulation various pharmacological effects are thought to depend on the inhibition of the uptake of specific monoamine neurotransmitters (Ritz et al 1987).

The present study was undertaken to investigate the effect of trans-[Co(3-R-pyridine)$_4$Cl$_2$]Cl, "since R is methyl or ethyl" on the rabbit brain MAO activity. This compound is similar (in structure) to phenyl pyridine and phenyl piperidine which used in the treatment of depression, so this study may add some information on the enzyme mechanisms of action and on the use of the inhibitors or activators in the clinical field.

Materials and Methods

Preparation of the Complexes

Trans-[Co(3-methy pyridine)$_4$Cl$_2$]Cl "CMPC" and trans-[Co (3-ethyl pyyridine)$_4$Cl$_2$] Cl "CEPC" were prepared by the method of Werner and Feenstra (1906) as modified by Elgy and Wells (1980) where, 23.79 gms of $CoCl_2$. $6H_2O$ were dissolved in 100 ml water, 37.25 gms of 3-methyl-or 3-ethylpyridine were dropped in the $CoCl_2$ solution with continuous stirring, eventually a pink paste was obtained. Chlorine gas was passed through this paste for several hours till the colour of the mixture turned to dark green, and then green crystals was formed during the passage of the chlorine gas through the solution. The solid was filtered and recrystalized from hot water and reprecipitated by adding a few drops of concentrated hydrochloric acid. The complex was filtered and washed with cold water and then dried in a vaccuum desiccator. Correct chemical identify for methyl and ethyl complex products purity by using UV spectra, IR spectra, NMR-spectra and FAB-spectra were carried out.

Preparation of Rabbit Brain MAO

Rabbits (weight about 1.5 kg, N=4) were decapitated and allowed to bleed, the brain were removed as quickly as possible, blotted on filter paper and calculate its weight. All subsequent procedures were performed at 0-4^{o}C.The brains were homogenized

in four volumes (w/v) of 0.25 M sucrose, 0.1 M sodium phosphate buffer (pH 7.4) in a Teflon glass homogenizer. The homogenates were centrifuged at 600 g. for ten minutes. The supernatant fraction was devided into 3 ml portions in small screw cap vials and kept frozen at -30°C for latter assaying of MAO.

Enzyme Assay

Activity of the enzyme (toward benzylamine was determined according to the method of Tabor (1954) with a reaction mixture containing 10 uM of benzylamine-HCl, (3-x) ml of sodium phosphate buffer, pH 7.4, and x ml of enzyme. The reaction was started by adding the enzyme solution and the change in absorbance at 250 nm due to benzaldehyde formation was followed at 30°C with a Beckman model DB-G spectrophotometer.

One unit of activity is defined as the amount of enzyme catalyzing the formation of 1 uM of benzaldehyde/min under the assay conditions described. The amount of the product was calculated from the molar extinction coefficient (E_{250nm} = 12,800 M^{-1} (min^{-1}).

Protein content of the enzyme was determined by the method of modified Lowry (Ohnishi and Parr, 1978).

Results

Effect of (CMPC) on rabbit brain MAO

This study demonstrates that CMPC complex inhibit MAO enzyme since the benzylamine was used as substrate. The inhibition of MAO was measured under conditions of varying preincubation time (zero to 30 minutes), since the concentration of CMPC compound was 2.5×10^{-9}M. It was found that the inhibition increased with increasing the preincubation time (fig. 1) and also the degree of MAO inhibition attained after the preincubation period varied with the concentration of CMPC. ($2x10^{-12}$ M to $3.3x10^{-8}$ M) with I_{50} value equal to $8.0x10^{-12}$M concentration (Fig. 2).

Fig (3) shows, the Lineweaver-Burk plot in the absence and presence of $8.3x10^{-10}$ M of CMPC. The V_{max} values for both cases are 222.2 nmole/mg protein/min and 71.4 nmole/mg protein min respectively, however the K_m values are the same value (17.6 uM) This mean that, CMPC inhibit the MAO by a noncompetitively inhibition type and $8.3x10^{-10}$ M cause 68% inhibition.

Effect of (CEPC) on rabbit brain MAO

MAO activities were determined under the effect of CEPC, since benzylamine was used as substrate. The concentration of CEPC complex ranging from $2x10^{-12}$M tc $3.3x10^{-8}$ M were used. These studies illustrated that CEPC inhibit the production of

benzaldehyde and this inhibition increased with increasing the CEPC concentration (Fig. 2).

It was found also that the MAO inhibition was clearly time dependent through the entire time course of the experiment (fig.1).

Fig. (3) illustrates, the lineweaver-Burk plot without or with $8.3x10^{-10}$ M of CEPC complex. The values of V_{max} in both cases are equal to 222.2 nmole/mg prot/min and 57.1 nmole/mg protein/min respectively, however the K_m values are 17.6 uM. According to this result, we concluded that, the CEPC inhibit the MAO by a noncompetitively inhibition type, since $8.3x10^{-10}$M cause 74% inhibition.

Effect of methylpyridine on MAO activity

MAO activity was measured under the effect of Metnylpyridine which is a precursor taking part in the constituents of the cobalt pyridine complex. The enzyme was inhibited by methyl-pyridine and this inhibition was found increase by increasing the concentration of methyl pyridine (fig. 4).

Discussion

This study illustrates that, the oxidative deamination of benzylamine by MAO was inhibited by the cobalt pyridine complex.

The inhibition of enzyme by this complex was increased by preincubation time and the concentration of the inhibitor.

We investigated the interaction of CMPC and CEPC with a rabbit MAO since benzylamine was used as substrate. The data indicate that these compounds were a noncompetitive inhibitors of MAO. $8.3x10^{-10}$ M of CMPC inhibited the benzylamine deamination 68%, wherease $8.3x10^{-10}$ M of CEPC inhibited 74% of deamination of benzylamine. In other words four time the concentration of the simple methyl pyridine was equavalent to one concentration of the CMPC complex were found to give almost equal 68% inhibition.

The active site of MAO has been suggested to be composed of two segments. One segment bears the FAD prosthetic group, and the other segment comprises the amino acid residues of the substrate binding site. The amino acid sequence analysis of the FAD peptide [Kearney et al, 1971; Nagy and Salach, 1981; Walker et al, 1971; Yu, 1981], isolated after complete proteolytic digestion of purified beef liver MAO-B and human placenta-MAO-A, has revealed that the FAD is linked through the cysteine residue to the same penta peptide (Ser-Gly-Gly-Tyr) for both MAO-A and MAO-B.

The results indicate that the trans-[Co(3-methyl pyridine)$_4$ Cl_2]Cl "CMPC" and trans-[Co(3-ethyl pyridine)$_4$

Cl_2]Cl "CEPC" are not bound at the substrate binding site but at some other binding site on the enzyme which indicated from the structure activity relationship between the simple methyl pyridine and the cobalt pyridine complex, CMPC (and also between CMPC and CEPC) interact with enzyme substrate hydrolyse activities. It may bound at the FAD binding site.

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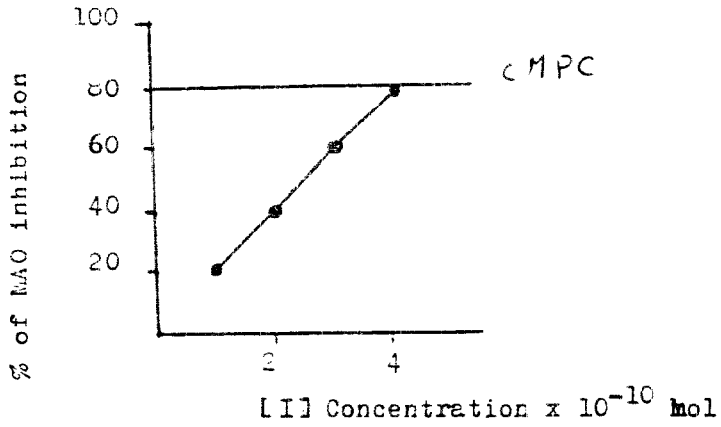

Fig. 4 : Effect of different concentration of methyl pyridine and 0.3x10-10 of CMPC.on MAO activity.

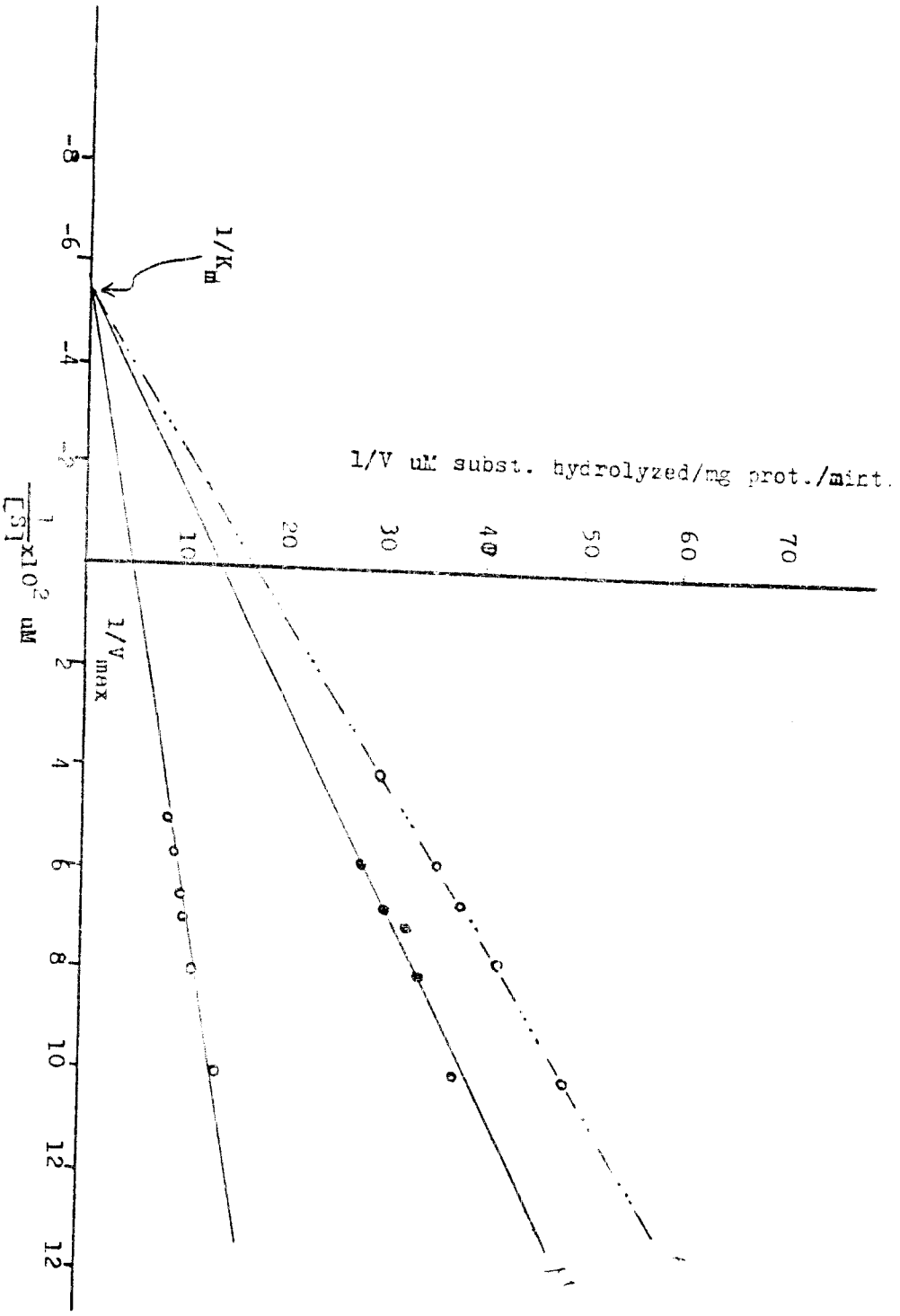

Fig. 3 : Lineweaver-Burk plot 1/v versus 1/S in the absence o—o and presence of 8.3x10-10 M of CMPC •—• and 8.3x10-10 M of CEPC o--o

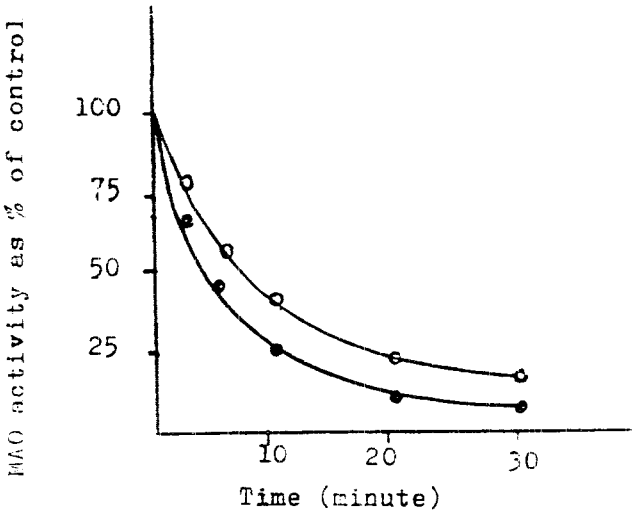

Fig. 1 : Effect of preincubation time upon inhibition of MAO activity by o—o CMPC and CPEC •—• 8.3 uM of benzylamine was used as substrate.

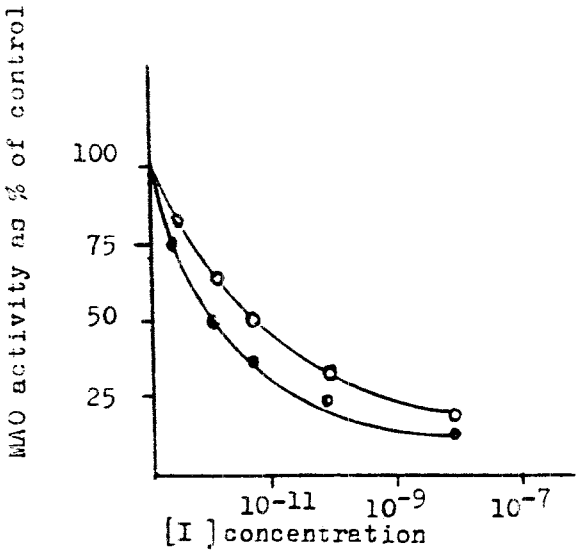

Fig. 2 : Effect of different concentration of CMPC o—o and CEPC •—• compounds on the MAO activity 8.3 uM of benzylamine was used as substrate.

تثبيط احادى امين الاكسيداز بمخ الارانب
بواسطة متراكبات ثنائى كلور الكوبلت
الثلاثى لمشتقات البريديـــن

عادله زكى شعبان
قسم الكيمياء الحيوية

جيهان مصطفى الصرونـى
قسم الكيميـــاء

كلية العلوم ـ جامعة الاسكندرية

المخلص العربى

ترجع اهميه هذا البحث ان هذه المتراكبات شبيهة بمركبات فنيل بيريدين ، وفنيل بيريدين والتى ستخـــدم فى علاج الاكتئاب ولذلك فما بدراسه تاثير هذه المتراكبات

أ ـ
٢ ـ
trans - Co (3- methylpyridine)$_4$Cl$_2$) Cl
trans - Co (3- ethylpyridine)$_4$ Cl$_2$) Cl

على نشاط انزيم احادى امين الاكسيداز حيث ان المنشطات او المعوقات للانزيم يمكن ان يستفاد بها فى المجـــال الاكلينيكـى .

تم دراسة نشاط انزيم MAO تحت الظروف المناسبة له والتى اظهرت نشاط طبيعى معتمدا على تركيز البروتين الانزيمى نيعطى اعلى نشاط يساوى ٢٢٢ر٣ نانو مول من ماده التفاعل متحلله ولكل ١ مجم بروتين انزيمى لكل دقيقه

اظهر المركب رقم (١) قوه تثبيطيه للانزيم معتمدا على تركيز المركب رقم (١) المستخدم يظهر من قيمه التركيز العقل لتثبيط ٥٠٪ من نشاط الانزيم التى ص٨×١٠$^{-12}$ مولار كذلك قيم ثابت ميخائيل للانزيم فى وجود تركيز المركب رقم (١) او الانزيم بمفرده كانت ٧ر١٧ميكرومول فى تثبيط غير تنافسى مع تركيز ماده التفاعل . واعلى نشاط للانزيم فى وجود ٨×١٠$^{-10}$ من تركيز المركب رقم (١) كان ٤ر٧١ نانو مول ماده تفاعل لكل ١ مجم بروتين انزيمى لكل دقيقه .

كما اوضحت النتائج ان المركب رقم (٢) ينشط نشاط الانزيم ايضا بطريقه مشابهه مع زياده التركيز بعد عمليه التحفيز للبروتين الانزيمى وتركيز المركب رقم (٢) المستخدم .

وقد اظهرت النتائج ان تركيز المركب رقم (٢) المنشط ل ٥٠٪ من النشاط الانزيمى كانت ٣×١٠$^{-11}$ مولـــر وقد ينشط هذا المركب الانزيم فى تنشيط غير تنافسى مع ماده التفاعل بثوابت تنشيط مقدارها ٧ر١٧ ميكرومول لثابـــت ميخائيل واعلى نشاط تنشيطى عند تركيز ٨×١٠$^{-10}$ مول كانت ٥٧ر١ نانومول ماده تفاعل متحلله لكل ١ مجم بروتين انزيمى لكل دقيقه .